

Gene Section

Review

MIRN21 (microRNA 21)

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Identity

Hugo: MIRN21

Other names: hsa-mir-21; miR-21

Location: 17q23.1

Location base pair: MIRN21 is located on chr17: 55273409-55273480 (+).

Local order: Based on Mapviewer, genes flanking MIRN21 oriented from centromere to telomere on 17q23 are:

- TMEM49, transmembrane protein 49, 17q23.1.
- MIRN21, microRNA 21, 17q23.1.
- TUBD1, tubulin, delta 1, 17q23.1.
- LOC729565, similar to NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8, 19 kDa, 17q23.1.
- RPS6KB1, ribosomal protein S6 kinase, 70 kDa, polypeptide 1, 17q23.1.

DNA/RNA

Description

The gene is located in an intergenic region. The length of MIRN21 gene is reported as 3433 nucleotides long. It overlaps with the 3' UTR end of the Transmembrane Protein 49 (TMEM 49) (also known as Human Vacuole Membrane Protein 1, VMP-1).

Transcription

RNA Pol II is suggested to be the most likely enzyme involved in miRNA transcription. However, current studies also provide evidences for RNA Pol III dependent transcription of few miRNAs interspersed among repetitive Alu elements.

For MIRN21, the major RNA polymerase is likely to be RNA Pol II due to the presence of 5' cap and 3' poly (A) tail of the pri-MIRN21. Chromatin immunoprecipitation (ChIP) analysis of upstream

sequences of MIRN21 showed enrichment for Pol II but not Pol III.

MIRN21 gene was shown to harbor a 5' promoter element. 1008 bp DNA fragment for MIRN21 gene was cloned (-959 to +49 relative to T1 transcription site, see Figure 1; A). Analysis of the sequence showed a candidate 'CCAAT' box transcription control element located approximately about 200 nt upstream of the T1 site. T1 transcription site was found to be located in a sequence similar to 'TATA' box (ATAAACCAAGGCTCTTACCATAGCTG). To test the activity of the element, about 1kb DNA fragment was inserted into the 5' end of firefly luciferase indicator gene and transfected into 293T cells. The sense orientation insert, unlike antisense, induced luciferase activity.

pri-MIRN21 gene was reported to have two transcription sites, T1 and T2. T1 (identified by RACE, +1 start site) was reported as the minor transcription site and T2 (identified by RACE, +27 start site) as the major transcription start site. Based on the data of pmiR-21-luc expression plasmid, the endogenous pri-MIRN21 was suggested to utilize T1 and T2 sites for initiation of transcription (Figure 1; A).

The maturation of miRNA gene involves sequential process.

Pri-miRNA

The miRNA genes are first transcribed in nucleus as long primary transcripts called pri-miRNA. The primary transcript for MIRN21 is found to be 3433-nt long.

For localization of the pri-MIRN21 transcript, total, nuclear and cytoplasmic RNA fractions from HeLa cells were oligo-dT primed and reverse transcribed into cDNA. pri-MIRN21 transcript was found mainly in the nucleus as well as modest levels in the cytoplasm.

Sequence: NCBI cDNA clone: BC053563. Length: 3389bp

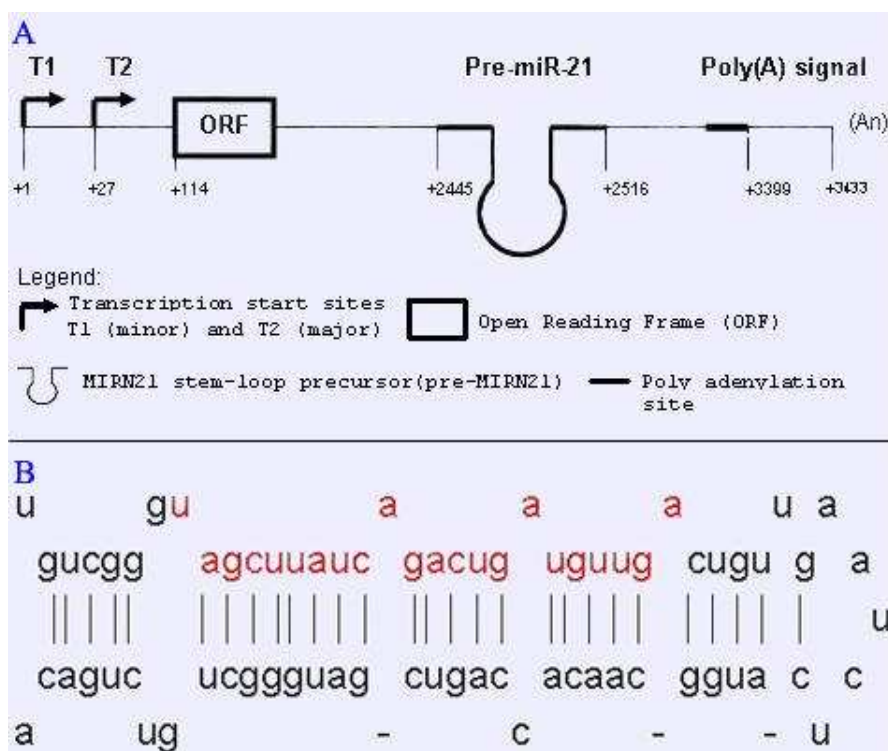


Figure 1. A: Characterization of the full-length about 3433 nt pri-MIRN21.

Open Reading frame analysis within the 3433 nucleotides identified a potential 124 amino acids long peptide. This uncharacterized ORF is located near the transcription start site (+114).

This potential peptide sequence shows homology to a 180-amino-acid human protein. However, it is not clear yet if pri-MIRN21 functions as an mRNA as well.

Figure 1. B: Stem-loop structure of MIRN21.

Pre-miRNA

The primary transcripts of microRNAs are processed by enzymatic microprocessor Drosha (RNase III enzyme) and DGCR8 (dsRNA binding protein) from their 3' and 5' cleavage sites into an intermediate stem-loop precursor or pre-miRNA in the nucleus.

The precursor of MIRN21 is 72 bases long (pre-MIRN21), forms a secondary structure, and contains the mature miRNA sequence, stem and terminal loop structures with 2-nt 3'overhang (Figure 1; B). The precursor is then transferred from nucleus to cytoplasm by the enzyme Exportin 5. In cytoplasm, a second RNase III enzyme, Dicer, removes terminal loop generating about 20-bp RNA duplex.

Length: 72 bases

Sequence:

UGUCGGGUAGCUUAUCAGACUGAUGUUGACU
GUUGAAUCUCAUGGCAACACCAGUCGAUGGG
CUGUCUGACA (Figure 1; B).

Mature MIRN21

The mature miRNA forms one strand of the RNA duplex. One strand is degraded and other is

incorporated in to a protein complex, RNA induced silencing complex (RISC), targeting a partially complementary target mRNA.

MIRN21 is 22 nucleotides long.

Sequence: UAGCUUAUCAGACUGAUGUUGA.

Pseudogene

No reported pseudogenes.

Protein

Note: miRNAs are not translated into amino acids.

Mutations

Note: In a panel of 91 human cancer cell lines representing several human cancers, sequencing showed no sequence variations in mature miRNAs.

In HCT-15 colon cancer cell line, pri-MIRN21 showed a A+29G (A/G) heterozygous variation (Figure 2).

It was suggested that sequence variations in pri-miRNAs may cause structural alterations. However, the variation was not found to be affecting pri-MIRN21 processing when it was compared to the wild type.

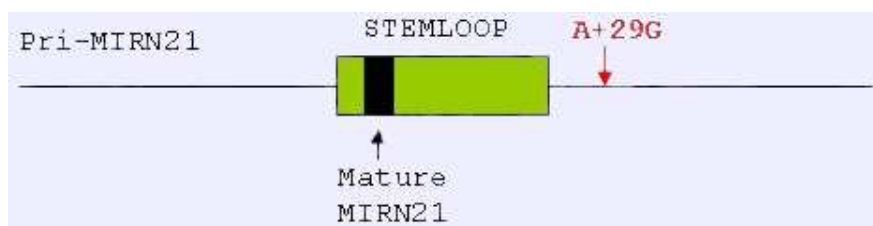


Figure 2. Localization of sequence variation in pri-MIRN21 in HTC-15 colon cancer cell line.

Implicated in

Human neoplasms

Note: Overexpression was first shown in glioblastoma and then in papillary thyroid carcinoma (PTC), breast tumors and other various tumors (e.g. colorectal carcinoma, lung tumors, pancreatic tumors, prostate tumors, stomach tumors, cholangiocarcinomas, neuroblastoma, hepatocellular carcinoma and uterine leiomyomas) and cervical adenocarcinoma cell line, HeLa.

Relatively low expression was seen in cell lines HL-60 (promyelocytic leukemia), K562 (chronic myelogenous leukemia) and prostatic adenocarcinoma cell line.

miRNA microarray data from 540 samples from 6 solid cancers (lung, stomach, prostate, colon, pancreatic and breast) showed overexpression of MIRN21 gene compared to normal cells.

Glioblastoma

Disease

Overexpression of MIRN21 was first shown in malignant human brain tumor cells. When, human glioblastoma tumor tissues, 12 early passage cultures (passage 3) from high grade gliomas and 6 glioblastoma cell lines (A172, U87, U373, LN229, LN428 and LN308) were compared to non-neoplastic glial cells and a variety of mammalian tissues, MIRN21 was found to be strongly overexpressed in the neoplastic samples. Moreover, oligonucleotide microarrays specific for 180 human and mouse miRNAs and Northern blotting methods were used to profile expression of MIRN21. In glioblastoma tissues its expression showed 5 to 100 fold increase compared to non-neoplastic brain sample and 5 to 30 fold increase in cell lines compared to normal.

Oncogenesis

Apoptosis: Loss-of-function approach was used to identify the biological significance of MIRN21 in glioblastoma cells. Sequence specific inhibitors (2'-O-methyl-oligonucleotides) were used to knock-down MIRN21 transcript and apoptosis activity (caspase-3 and caspase-7 enzymatic activities) was measured. 48 hours post-transfection, caspase activity increased 3-folds suggesting that MIRN21 acted as an anti-apoptotic factor in glioblastoma cells through blocking expression of key apoptosis-enabling genes.

Breast Cancer

Disease

RNAs from 76 breast cancer tumors and 14 cell lines were analyzed by using miRNA microarray and Northern blotting (10 normal samples were used for comparison and normalization). MIRN21 was up-regulated and the results were confirmed by Northern blotting.

Consistent with other studies, MIRN21 overexpression in breast tumors compared to matched normal breast tissues was verified by stem-loop RT real-time PCR and miRNA microarrays containing 157 mature human miRNAs.

Oncogenesis

Apoptosis: Inhibition of MIRN21 in breast cancer cell line MCF-7 by transfection of anti-mir-21 inhibitors (chemically modified oligonucleotides) showed growth inhibition. Treatment of transfected MCF-7 cell line with anticancer drug topotecan (TPT) caused cell growth inhibition by 40%. The results suggested suppression of MIRN21 gene could sensitize tumor cells to anticancer drugs. Inhibition of MIRN21 in a xenograft carcinoma mouse model verified tumor growth suppression.

Transfection results of MCF-7 cells with a general caspase inhibitor suggested MIRN21 role in regulation of bcl-2 gene expression indirectly, possibly controlling expression of genes involved in apoptosis pathways including bcl-2.

Pancreatic cancer

Disease

16 pancreatic adenocarcinomas and 10 adjacent benign tissues compared to 6 normal pancreas samples were analyzed for MIRN21 precursor expression and compared to mature MIRN21 by using real-time PCR assay. The results were consistent between precursor and mature MIRN21 showing overexpression.

Neuroblastoma

Disease

Neuroblastoma cell line, SH-SY5Y, was treated with a tumor promoting agent (12-O-tetradecanoyl phorbol 13-acetate (TPA)) to induce differentiation into a neuronal phenotype. Following stimulation, microarray analysis of stem-loop precursors was performed and MIRN21 showed 7-8 times higher expression

compared to other up-regulated miRNAs showing 2-4 times relative increase.

Lung cancer

Disease

Analysis of 104 pairs of primary lung cancers and non-cancerous lung tissues by microRNA microarray showed differential expression of mature MIRN21 among phenotypical and histological classifications. The results were confirmed by solution hybridization and RT-PCR. The results verified up-regulation of MIRN21 in lung cancer tissues compared to normals. Moreover, real time RT-PCR results for stem-loop precursor of MIRN21 showed at least 2-fold up-regulation in 66% of 32 cases.

Other cancers

Disease

In other miRNA microarray studies, MIRN21 was found to be overexpressed in papillary thyroid cancer, hepatocellular carcinoma, cholangiocarcinomas and uterine leiomyomas. A study suggested that MIRN21 inhibition in a cervical adenocarcinoma cell line, HeLa, caused increase in cell growth.

Prognosis

MIRN21 (as well as 7 other miRNAs) expression was correlated with adenocarcinoma patients' survival. Patients that have high expression of MIRN21 were found to have worse prognosis. Thus, in addition to potential role of MIRN21 in lung carcinogenesis through apoptosis pathway, it was suggested that expression profiles could be informative in adenocarcinoma patient survival.

Cytogenetics

Genomic amplification of chromosome band 17q23.2 in neuroblastoma, breast cancer, colon cancer, lung cancer is known.

Oncogenesis

Apoptosis: MIRN21 was found to be highly over-expressed in malignant cholangiocytes. In cholangiocarcinoma cells it was shown that one of the targets of MIRN21 was PTEN encoding phosphatase that inhibited the survival and growth promoting activity of PI 3-kinase (phosphoinositole 3-kinase) signaling.

In another report, inhibition of MIRN21 showed increased sensitivity to gemcitabine. The results suggested that MIRN21 regulated gemcitabine-induced apoptosis by PTEN (phosphatase and tensin homolog) dependent activation of PI 3-kinase and AKT/mTOR signaling. These studies suggested anti-apoptotic role for the MIRN21 gene.

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